Mutation profile and clinical outcome of mixed endometrioid-serous endometrial carcinomas are different from that of pure endometrioid or serous carcinomas

Coenegrachts L.[1*], Garcia-Dios D.A.[1,2*], Depreeuw J.,[1,2,3] Santacana M. [4], Gatius S[4], Zikan M.[5], Moerman P.[6], Verbist L.[1], Lambrechts D.[2,3], Matias-Guiu X.[4*], Amant F.[1,7*].

[1]Gynecologic Oncology, Department of Oncology, KULeuven, Belgium; [2]Laboratory for Translational Genetics, Vesalius Research Center, VIB, KU Leuven, Leuven, Belgium; [3]Vesalius Research Center, Leuven, VIB, Belgium; [4]Department of Pathology and Molecular Genetics and Research Laboratory, Hospital Universitari Arnau de Vilanova, University of Lleida, IRBLLEIDA, Lleida, Spain; [5]Gynecological Oncology Centre, Department of Obstetrics and Gynecology, First Faculty of Medicine and General University Hospital, Charles University in Prague, Czech Republic; [6]Department of Pathology. University Hospitals Gasthuisberg, Leuven, Belgium; [7]Obstetrics & Gynecology, Division of Gynecologic Oncology, University Hospitals Gasthuisberg, Leuven, Belgium

*Equal contribution

Corresponding authors:

Frédéric Amant
Gynecologic Oncology - University Hospital Leuven
Herestraat 49
B-3000, Leuven, BELGIUM
frederic.amant@uzleuven.be
Phone: +32 (0)16 344635
Fax: +32 (0)16 344629

Xavier Matias-Guiu
Pathology and Molecular Genetics,
Institut de Recerca Biomedica de Lleida
Alcalde Rovira Roure 80
25198 Lleida, Spain
fjmatiasguiu.lleida.ics@gencat.cat
Phone +34 973705340

Key words: Endometrial cancer, mixed endometrial carcinomas, mutation analysis, clinical outcome

Abstract

Clinical outcome of 23 patients with mixed endometrioid and serous endometrial carcinomas (mixed EEC-SC) was compared to that of pure endometrioid (EEC) and pure serous (SC) carcinomas. Hotspot mutation frequencies in *KRAS, PIK3CA, PTEN* and *TP53* and microsatellite instability (MSI) status were determined in mixed EEC-SC, as well as in their EEC and SC micro-disected components separately, and alterations were compared to frequencies in pure EEC and SC. Relapse-free (RFS) and overall survival (OS) differed significantly between mixed EEC-SC and pure EEC and SC, revealing that outcome of mixed EEC-SCs was intermediate to that of pure EEC and pure SC. *PTEN* mutations were absent in pure SC, but occurred in 20% of pure EEC, and 13% of mixed EEC-SC. In contrast, *TP53* mutations were more frequent in pure SC (17%) and mixed EEC-SC (22%) than in pure EEC (2%). Mutations in mixed EEC-SC were shared by the two micro-dissected components in 30 %, whereas in 35% some mutations were component-specific. Mutation analysis confirms similarities between the EEC and SC components of mixed EEC-SC with pure EEC and pure SC respectively. However, *PTEN* and *KRAS* mutations were more frequent in the SC component of mixed EEC-SC than in pure SC, while *TP53* mutations were more frequent in the EEC component of mixed EEC-SC than in pure EEC. Presence of different clonal mutation pattern between EEC and SC components of mixed EEC-SC raises the possibility of divergent tumor heterogeneity or biclonal origin in some cases.

Introduction

In industrialized countries, endometrial carcinoma is the most frequent gynaecological cancer [1]. Although 75% of these patients are diagnosed in an early stage and have favorable outcome, near 20% of the cases will recur with dismal prognosis. Based on pathologic characteristics, the majority of the cases can be divided into two different groups, type I and type II endometrial tumors. Type I tumors correspond to endometrioid endometrial carcinoma (EEC) and present a favorable prognosis. On the other hand, type II or non-endometrioid endometrial carcinoma (NEEC) corresponds to high-grade disease and frequently correlates with increased risk for relapse and bad prognosis [2]. Serous carcinoma (SC) is the paradigm for NEEC. However, not all endometrial tumors can be included into one of the above-mentioned subtypes. Approximately 3-10% of the cases present overlapping microscopic features of two or more histologic subtypes. When one of these components is present

in at least 5% of the tumor, the tumor is diagnosed as a mixed endometrial carcinoma, mixed endometrioid and serous carcinoma (mixed EEC-SC) being the most frequent tumor type. The correct diagnosis of the second component is crucial to determine treatment options and outcome for these patients, since it has been suggested that the presence of type II component regardless of the amount might adversely affect patient outcome [3]. Conversely, other studies have shown a more favourable outcome for patients with a mixed endometrial tumor as compared to patients with a pure type II tumor [4].

The clinico-pathological and etiological aspects of mixed endometrial tumors have been poorly studied, mainly due to their relative rarity. Based on molecular analysis, it has been suggested that the NEEC component originates as a result of tumor progression from a pre-existing EEC, because frequently these tumors retain molecular alterations of typical EEC tumors [2]. Furthermore, studies investigating the mutation profile of each component individually are rare and usually report on a small number of samples (n<5) [5]. Nevertheless, these molecular analyses are crucial to gain more insight into the pathogenesis of mixed endometrial tumors, which might help in developing better prognostic indicators for mixed endometrial cancer patients.

In this collaborative multicenter study, we collected a series of 23 mixed EEC-SC, assessed the prognosis of mixed EEC-SC in comparison with a previous series of pure EEC and pure SC, and performed detailed molecular analysis on the separate components by checking hot spot mutations in four genes that are frequently altered in endometrial cancer. Moreover, we also determined the microsatellite instability (MSI) status of these tumors and their separate micro-dissected components.

Material and Methods

Patients

Since mixed EEC-SC are rare, we established a multi-center international collaboration and collected formalin-fixed paraffin-embedded (FFPE) tumor tissues from 23 patients from the University Hospitals Leuven, Belgium, Arnau de Vilanova University Hospital, Lleida, Spain, and General Faculty Hospital, Prague, Czech Republic. All tumors were diagnosed as mixed EEC-SC, as they contained an endometrioid (EEC) and a serous (SC) component. In all cases, the minor component represented at least 5% of the tumor, in agreement with the more recent WHO classification [6]. The proportion of the serous component ranged from 10% to 90% (mean 52%). In order to compare survival and mutation frequencies with tumors of a single morphotype, a set of 24 pure SC and 230 pure EEC from the University Hospitals Leuven were included in the study. These tumors were genotyped in a previously published study by our group [7]. The series of 24 pure SC and 230 pure EEC were reassessed histologically to exclude mixed EEC-SC. For each case (of mixed EEC-SC, pure SC and pure EEC) clinical data (including staging, treatment and follow-up), pathology report, and paraffin-embedded blocks were available. The current study obtained the relevant IRB approval in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from each patient. All patients were subjected to similar strategies of surgical staging. EEC patients were treated according to the estimated risk of recurrence, whereas SC and mixed EEC-SC patients were treated with similar adjuvant systemic approaches.

Pathology

To identify the different components of each mixed EEC-SC, paraffin sections of all tumors were critically reviewed by a central group of expert pathologists (M.S, S.G. and X. M-G.). Agreement between the original and the central group diagnosis was an inclusion requirement (Figure 1). In particular, based on hematoxylin and eosin (H&E) staining andimmunohistochemical staining for p53 (tumor protein 53, serous), insulin-like growth factor 2 (IMP2; serous) and estrogen receptor (ER; endometrioid carcinoma) [8, 9], the different components were identified and a representative tumor area suitable for DNA extraction of each of the different components was selected. In some cases, the immunohistochemistry panel also included PTEN, and p16 (Figure 1B).

DNA extraction and somatic oncogene profiling

After pathological examination, 1-10 FFPE slides (5-10 µm) were used for DNA extraction. Tumor tissue was macro-dissected from individual slides, under microscopic control. The different EEC and SC components from each tumor were separated before deparaffinization. After deparaffinization and tissue digestion, DNA was extracted using a phenol/chloroform protocol as described before [7]. In total, we retrieved sufficient DNA from both components of 23 mixed EEC-SC tumors.

Tumors were genotyped by using the MassARRAY Compact Analyser (Sequenom Inc., San Diego, USA), as described previously [7]. Briefly, automated genotyping calls were generated using the MassARRAY RTTM software and validated by manual review of the mass spectra. Mutations in different genes mainly belonging to the PI3K/AKT/PTEN/mTOR pathway were included in the study. Hotspot mutations in *PIK3CA*, *KRAS*, *PTEN*, and *TP53*, were genotyped, respectively 21, 33, 11, and 18, somatic mutations that cover, 98%, 81%, 14%, and 16% of all somatic mutations occurring in these above mentioned genes, as reported in the COSMIC (Catalogue Of Somatic Mutations In Cancer database) [10]. It is important to note that our genotyping approach was designed to identify mutations in hot spot regions, which explains the expected lower frequency of mutations in *PTEN* and *TP53* in comparison with other series of cases which assessed the entire coding sequence of these genes. However, this approach is appropriate for a comparison between different tumors, or different components of each tumor, because for each of them the same methodology is used.

To determine the microsatellite instability status (MSI) of the separate components, we used a recently established 59-marker panel based on recurrent indels in MSI tumors to identify MSI [11]. In this panel, 59 recurrent indels are evaluated and when 3 or more positive markers are identified, a tumor can be categorized as MSI.

Statistical analyses and correlation with clinical data

Data are summarized as frequencies and percentages for categorical variables. Age at diagnosis was analysed as a binary variable (< or ≥60 years). Pearson's chi square test was used to compare categorical variables. Kaplan Meier survival and Cox regression analyses for relapse-free survival (RFS) and overall survival (OS) were used to assess differences in clinical outcome, while correcting for age and FIGO stage. *P* values were two-tailed and $P<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS software v17.0 (SPSS Inc., Chicago, USA).

Regarding sample size and statistical power, the series analyzed in this retrospective, observational and clinical study included clinical data and samples from 277 patients, comprising 230 EEC (83%), 24 SC (9%) and 23

mixed EEC-SC (8%). The study achieved 80% statistical power to detect a minimal Hazard Ratio of 1.94 and 2.31 units respectively for RFS and OS, using a Cox regression model to assess the differences among the three groups analyzed.

Results

Clinical outcome of patients with mixed EEC-SC is different from patients with pure EEC or pure SC

Baseline characteristics of all patients with pure EEC, mixed EEC-SC and pure SC are given in Table 1. The median follow-up of all patients was 43 months (range, 0-142 months). Mean follow-up period was 41.3 months (range 1 to 101 months) for mixed EEC-SC, 40.8 months (range 4 to 112 months) for pure EEC, and 36.3 months (range 3 to 98 months) for SC patients. Overall, 230 patients and 24 patients were diagnosed with a pure EEC or pure SC respectively, whereas 23 patients had mixed EEC-SC. Pure SC were characterized by a higher risk for recurrence and a fatal outcome (dead-of-disease, DOD) (Table 1). The number of patients with recurrence was 3 for mixed EEC-SC, 34 for pure EEC, and 11 for pure SC. The number of patients with tumor related death was 2 for mixed EEC-SC, 18 for pure EEC, and 8 for pure SC.

To better assess the differences in clinical outcome between patients with mixed EEC-SC and pure EEC or pure SC, we next performed Kaplan-Meier survival analyses for relapse-free survivial (RFS) and overall survival (OS) (Figure 2A-B). These analyses showed significant differences in RFS and OS between the three patient groups (Log-Rank $P<0.001$). We therefore performed two different Cox regression models to assess statistical differences between the different patient groups (Table 2), with or without correcting for age and FIGO stage. In the first model using pure EEC patients as reference group, we observed that RFS and OS of only pure SC and not mixed EEC-SC were significantly different from these of pure EEC patients, also after adjustment for age and FIGO stage (Table 2). However, in the second Cox regression model using pure SC as reference group, no differences in RFS and OS were observed between mixed EEC-SC and pure SC after adjustment for age and FIGO stage (Table 2). These observations indicate that outcome of patients with mixed EEC-SC is intermediate between outcome of pure EEC and pure SC.

Mutation profile of mixed EEC-SC tumors is different from pure EEC and pure SC

Since clinical behaviour of mixed EEC-SC was different from pure EEC and pure SC, we next questioned whether this could be explained by a different genetic background of these tumors. Therefore, we first compared the mutation profile of mixed EEC-SC with that of pure EEC and pure SC. To this end, we selected 4 genes.

Two of them are frequently mutated in EEC (*KRAS* and *PTEN*), one is predominantly mutated in SC (*TP53*), while the fourth (*PIK3CA*) is frequently mutated in both EEC and SC. Briefly, screening of the COSMIC database [10], in combination with review of recent literature, resulted in a panel of frequent mutations in endometrial cancer that we used already in a previous study [7]. Fifty-six percent (13/23) of mixed EEC-SC tumors carried at least 1 mutation, whereas this was found in 38% (9/24) of pure SC and 47% (108/230) of pure EEC. Remarkably, mutations in *PIK3CA* and *KRAS* were more frequent in mixed EEC-SC (26%) than in pure EEC (13.5% and 17.0%) and pure SC (12.5% and 16.7%), although these differences were not significant. Mutations in *PTEN* were absent in pure SC whereas present in 19.6% of pure EEC and 13.0% of mixed EEC-SC (Pearson's Chi Square test P=0.047). *TP53* mutations were more frequent in mixed EEC-SC (22%) and pure SC (17%) than in pure EEC (1.7%) (Pearson's Chi Square test P<0.001; Table 3). In addition to the clinical outcome data, these mutation profiles also suggest that mixed EEC-SC share characteristics of both pure EEC and pure SC.

Mutation frequencies and MSI status in the separate tumor components of mixed EEC-SC tumors

When studying the mutation profiles of the separate micro-dissected tumor components in more detail, we observed that mutations detected in the mixed EEC-SC were shared by the two micro-dissected components in 30% (7/23) of all mixed tumors, whereas in 39% (8/23) of mixed EEC-SC tumors some mutations were component-specific. A detailed overview of the mutation patterns is given in Supplemental Table 1. Remarkably, mutation frequencies in the EEC and SC components were generally similar in both components (Table 4), but with some similarities with those found in the pure EEC and pure SC (also see Table 3). The frequency of *KRAS* and *PTEN* mutations in the EEC component of mixed EEC-SC (22% and 13%, respectively) was similar to that of pure EEC (17.0% and 19.6%), and higher than that of pure SC (13% and 0%). Moreover, the frequency of *TP53* mutations in the SC component of mixed EEC-SC (17%) was similar to that of pure SC (17%), and significantly higher than that of pure EEC (1.7%). Interestingly, the SC component of mixed EEC-SC had frequent mutations in *KRAS* and *PTEN* (17% and 9%), while the EEC component of mixed EEC-SC had frequent mutations in *TP53* (13%) (Table 4). Finally, we also determined the MSI status in the separate components of the tumors. Three mixed EEC-SC were MSI, in both components including the SC elements (Supplemental Table 2)

Discussion

Approximately 3-10% of all endometrial carcinomas are mixed, the majority composed of EEC and SC. In current practice, which follows recent 2014 WHO criteria, the presence of only 5% of either component is considered enough to categorize an endometrial carcinoma as mixed EEC-SC. It has been suggested that when a minor part of an endometrial carcinoma is composed of SC component, the patient has the same prognosis and risk for metastases as patients with pure SC [3]. Therefore, these patients are currently treated as patients with pure SC. However, in depth studies are rare and have not included any molecular data. In the present study we analysed both clinical outcome and molecular profile of mixed EEC-SC and compared these with pure EEC and pure SC. Since there is some interobserver variability in histological typing of mixed endometrial carcinomas, all tumors in our series of cases were histologically assessed by pathologists of the contributing center and by the central pathology group. Notably, pure EEC may exhibit papillary arrangements and may be erroneously diagnosed as SC. Conversely, some pure SC may show a glandular pattern of growth and may be misinterpreted as EEC. Inappropriate interpretation of these unusual pathological patterns may lead to incorrect diagnosis of mixed EEC-SC. The consensus diagnosis was supported by the results of immunohistochemical studies. Only cases with unambiguous consensus diagnosis were included in our study.

We found that survival of patients with mixed EEC-SC was not significantly different from that of patients with pure EEC or pure SC. In particular, survival of these patients was intermediate between survival of patients with pure EEC and pure SC. These observations are in contrast with those of previous studies [3], but support findings in other studies [4]. This emphasizes the importance of studies on large cohorts and strict pathologic inclusion criteria.

Our clinical observations raised the question whether the molecular profile of mixed EEC-SC might be different from that of pure EEC or pure SC. Therefore we first compared the mutation profile of mixed EEC-SC with that of pure EEC and pure SC. Mixed EEC-SC showed a higher frequency of *KRAS* (26%) and *PIK3CA* (26%) mutations than pure EEC (17% and 13.5% respectively) or pure SC (13% and 17% respectively), and this was also observed in the separate components of the tumors after microdissection. This indicates that mixed EEC-SC have a higher frequency of *KRAS* and *PIK3CA* mutations, regardless of the morphological appearance of the tumor component. This result confirmed previous observations of a higher frequency of *PIK3CA* mutations in mixed endometrioid-non-endometrioid carcinomas (44%), in comparison with pure non-endometrioid carcinomas (21%) and low- and high-grade EEC (26% and 34% respectively) [10].

By analysing the two components of mixed EEC-SC separately, we observed that these components show similarities with their corresponding pure tumor counterparts, but also some molecular ambiguities. For example,

the frequency of *KRAS* and *PTEN* mutations in the EEC component of mixed EEC-SC was comparable to that in pure EEC, whereas *TP53* mutations were much more frequent than in pure EEC. Moreover, the *TP53* mutation frequency in the SC component of mixed EEC-SC was comparable to that in pure SC, but a higher mutation frequency of *KRAS* and *PTEN* was observed in the SC component as compared to pure SC. These results emphasize the molecular ambiguity of the two components in mixed EEC-SC.

The separate analysis of the two components of a mixed EEC-SC has –to the best of our knowledge- never been described before and sheds new light on the biology of mixed endometrial tumors. Our observation that some mutations in mixed EEC-SC are shared by both components is in agreement with a previous hypothesis, suggesting that the serous component arises from a pre-existing EEC component [2]. However, the discrepancies we observed in the mutation pattern of the two components of some mixed EEC-SC, indicates that this hypothesis can not be generalized to all mixed endometrial tumors. Assuming that early alterations during carcinogenesis are shared by different components evolving later on, we postulate that mixed EEC-SC might not invariably be monoclonal but in some cases biclonal. Discrepancies in the mutation pattern between the two components of mixed EEC-SC might also be explained by the development of molecular heterogeneity during tumor progression.

Recently, the Cancer Genome Atlas Research Network (TCGA) has reported an integrating genomic characterization of endometrial carcinoma [13]. Results of this study show that by unsupervised hierarchical clustering of somatic copy number alterations 4 clusters of endometrial carcinomas emerge. One of these clusters, containing the majority of SC and mixed EEC-SC but also 12% of EEC (mainly grade 3), has a high degree of copy number alterations. More interesting, exome sequencing analsyis also revealed four groups of tumors (ultramutated, hypermutated, low copy number alterations – endometrioid, high copy number alterations – serous-like), each with a different prognosis. In this TCGA study only 13 mixed EEC-SC tumors were included, and there is no evidence that microdissection of the two components was performed. Only 62% of mixed EEC-SC had the molecular profile of serous-like tumors, emphasizing the molecular ambiguity of these tumors, which is in agreement with our results.

Understanding the molecular alterations of mixed EEC-SC is not only significant for prognostic purposes, but will allow better characterization and tailoring of targeted therapy for more personalized treatment. For example, our finding that mixed EEC-SC frequently show *PIK3CA* mutations suggests that PI3K- as well as m-TOR inhibitors may be an attractive treatment approach for this subset of patients [13, 14].

In conclusion, we show that mixed EEC-SC tumors are molecularly ambiguous, which corresponds with outcome of patients with mixed EEC-SC intermediate between that of patients with pure EEC and pure SC.

Acknowledgements

This work was supported by the Verelst Uterine Cancer Fund Leuven (VBL) and by a research grant of FWO Flanders (G.0827.13). This work is a result of collaboration within ENITEC (European Network for Individualised Treatment in Endometrial Cancer) as part of ESGO (European Society of Gynecological Oncology).

L.C. is a postdoctoral fellow of FWO Flanders, F.A. is senior researcher for FWO Flanders.

Conflict of interest

The authors declare that they have no conflict of interest

References

[1] Amant F, Moerman P, Neven P, Timmerman D, Van L.E, Vergote I (2005) Endometrial cancer. Lancet 366:491-505

[2] Yeramian A, Moreno-Bueno G, Dolcet X, Catasus L, Abal M, Colas E et al (2013) Endometrial carcinoma: molecular alterations involved in tumor development and progression. Oncogene 32:403-13

[3] Quddus M.R, Sung C.J, Zhang C, Lawrence W.D (2010) Minor serous and clear cell components adversely affect prognosis in "mixed-type" endometrial carcinomas: a clinicopathologic study of 36 stage-I cases. Reprod Sci 17:673-8

[4] Roelofsen T, van Ham M.A, Wiersma van Tilburg J.M, Zomer S.F., Bol M, Massuger L.F et al (2012) Pure compared with mixed serous endometrial carcinoma: two different entities? Obstet Gynecol 120:1371-81

[5] McConechy M.K, Ding J, Cheang M.C, Wiegand K.C, Senz J, Tone A.A et al (2012) Use of mutation profiles to refine the classification of endometrial carcinomas. J Pathol 228:20-30

[6] Kurman RJ, Carcangiu ML, Herrington CS, Young RH (2014) WHO classification of tumors of the female reproductive organs. WHO classification of tumors. IARC press, Lyon

[7] Garcia-Dios D.A, Lambrechts D, Coenegrachts L, Vandenput I, Capoen A, Webb P.M et al (2013) High-throughput interrogation of PIK3CA, PTEN, KRAS, FBXW7 and TP53 mutations in primary endometrial carcinoma. Gynecol Oncol 128:327-34

[8] Lax S.F, Kendall B, Tashiro H, Slebos R.J, Hedrick L (2000) The frequency of p53, K-ras mutations, and microsatellite instability differs in uterine endometrioid and serous carcinoma: evidence of distinct molecular genetic pathways. Cancer 88:814-24

[9] Zhang L, Liu Y, Hao S, Woda B.A, Lu D (2011) IMP2 expression distinguishes endometrioid from serous endometrial adenocarcinomas. Am J Surg Pathol 35:868-72

[10] Forbes S.A, Bindal N, Bamford S, Cole C, Kok C.Y, Beare D et al (2011) COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 39:D945-D950

[11] Catasus L., Gallardo A., Cuatrecasas M., Prat J (2009) Concomitant PI3K-AKT and p53 alterations in endometrial carcinomas are associated with poor prognosis. Mod Pathol 22:522-9

[12] Kandoth C, Schultz N, Cherniack A.D, Akbani R, Liu Y, Shen H et al (2013) Integrated genomic characterization of endometrial carcinoma. Nature 497:67-73

[13] Dedes K.J, Wetterskog D, Ashworth A, Kaye S.B, Reis-Filho J.S (2011) Emerging therapeutic targets in endometrial cancer. Nat Rev Clin Oncol 8:261-71

[14] Myers A.P (2013) New strategies in endometrial cancer: targeting the PI3K/mTOR pathway--the devil is in the details. Clin Cancer Res 19:5264-74

Tables and figures

Figure 1. Representative image of a mixed EEC-SC tumor (A). Representative immunohistochemical features (B).

Figure 2. Kaplan-Meier survival analysis for relapse-free surivival and overall survival of pure EEC, mixed EEC-SC and pure SC endometrial cancer patients. Abbreviations: EEC, endometrioid endometrial cancer; SC: serous endometrial cancer.

Table 1. Demographic and clinicopathologic characteristics of patients with pure EEC, mixed EEC-SC and pure SC.

Clinical data are stratified for pure EEC, mixed EEC-SC and pure SC. Percentages are given as column %. P values of a Pearson's Chi Square test are given. Abbreviations: EEC, endometrioid endometrial carcinoma; mixed EEC-SC: mixed endometrioid and serous carcinoma, SC: Serous carcinoma; NED; no evidence of

disease; AWED, alive with evidence of disease; DID, dead of intercurrent disease; DOD, dead of disease. Clinical variables were missing for some parameters and are indicated in the table.

Table 2: Cox Regression model proportional hazards (adjusted and unadjusted)

Cox regression analysis, whether or not adjusted for age and FIGO stage, was used to evaluate differences in RFS and OS in the three patient groups. In model 1, pure EEC was used as a reference, in model 2, pure SC was used as reference. Abbreviations: EEC: endometrioid endometrial cancer; SC: serous endometrial cancer

Table 3. Number of pure EEC, mixed EEC-SC and pure SC carrying at least 1 mutation in the indicated genes.

KRAS, *PIK3CA*, *PTEN*, and *TP53*, mutation frequencies respectively in pure EEC, mixed EEC-SC, and pure SC. Abbreviations: EEC, endometrioid endometrial carcinoma; Mixed EEC-SC: mixed endometrioid serous carcinoma; SC: Serous carcinoma. P values of a Pearson's chi square test are given.

Table 4. Hotspot mutation frequencies in separate components of mixed EEC-SC and pure EEC and pure SC.

KRAS, *PIK3CA*, *PTEN*, and *TP53*, mutation frequencies respectively in pure EEC, mixed EEC-SC, and pure SC. Abbreviations: EEC, endometrioid endometrial carcinoma; Mixed EEC-SC: mixed endometrioid serous carcinoma; SC: Serous carcinoma.

Supplementary Table 1. Detailed overview of all detected mutations in separate components of mixed EEC-SC. When a mutation is detected, this is indicated with 1, wild-type is indicated with 0. Colors are used to differentiate between mutations that are present in both components (green), mutations only in EEC component (yellow) and mutations only in SC component (orange).

Supplementary Table 2. Overview of MSI status of the separate components of the mixed EEC-SC. MSI status was determined using the 59-marker panel recently established by Zhao ET.AL. (under revision).

Figure
Click here to download high resolution image

Figure 1A

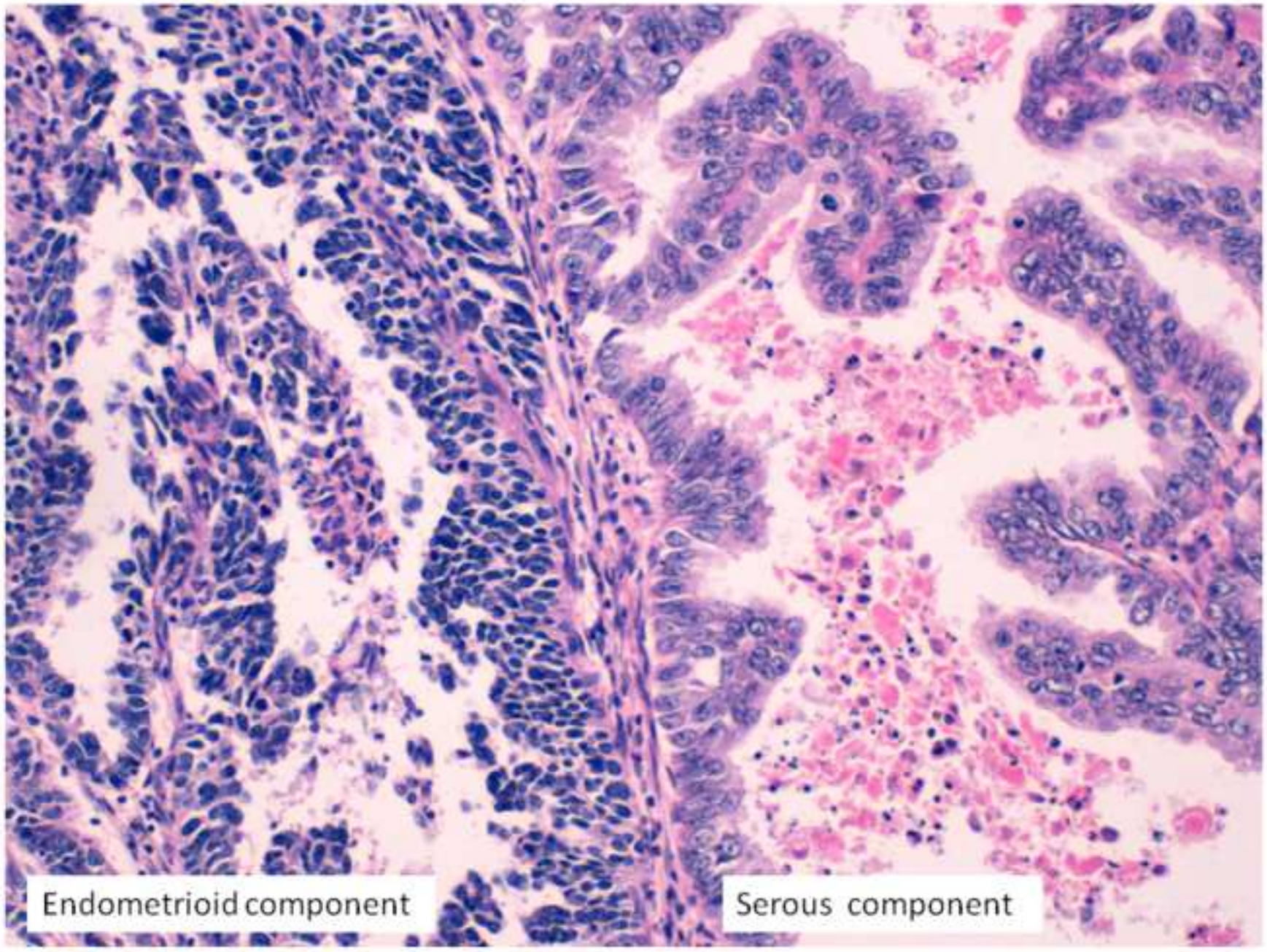


Figure
Click here to download high resolution image

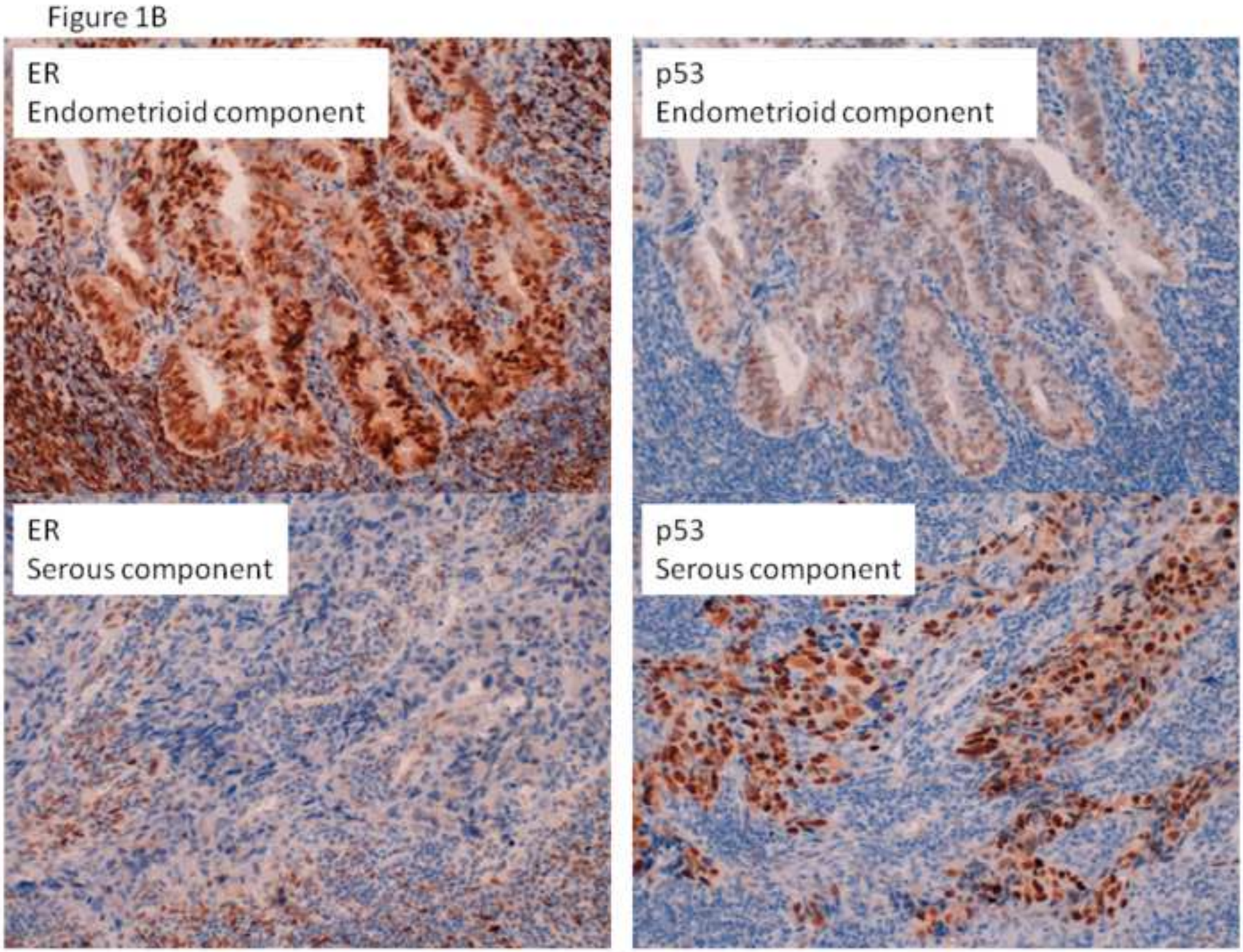
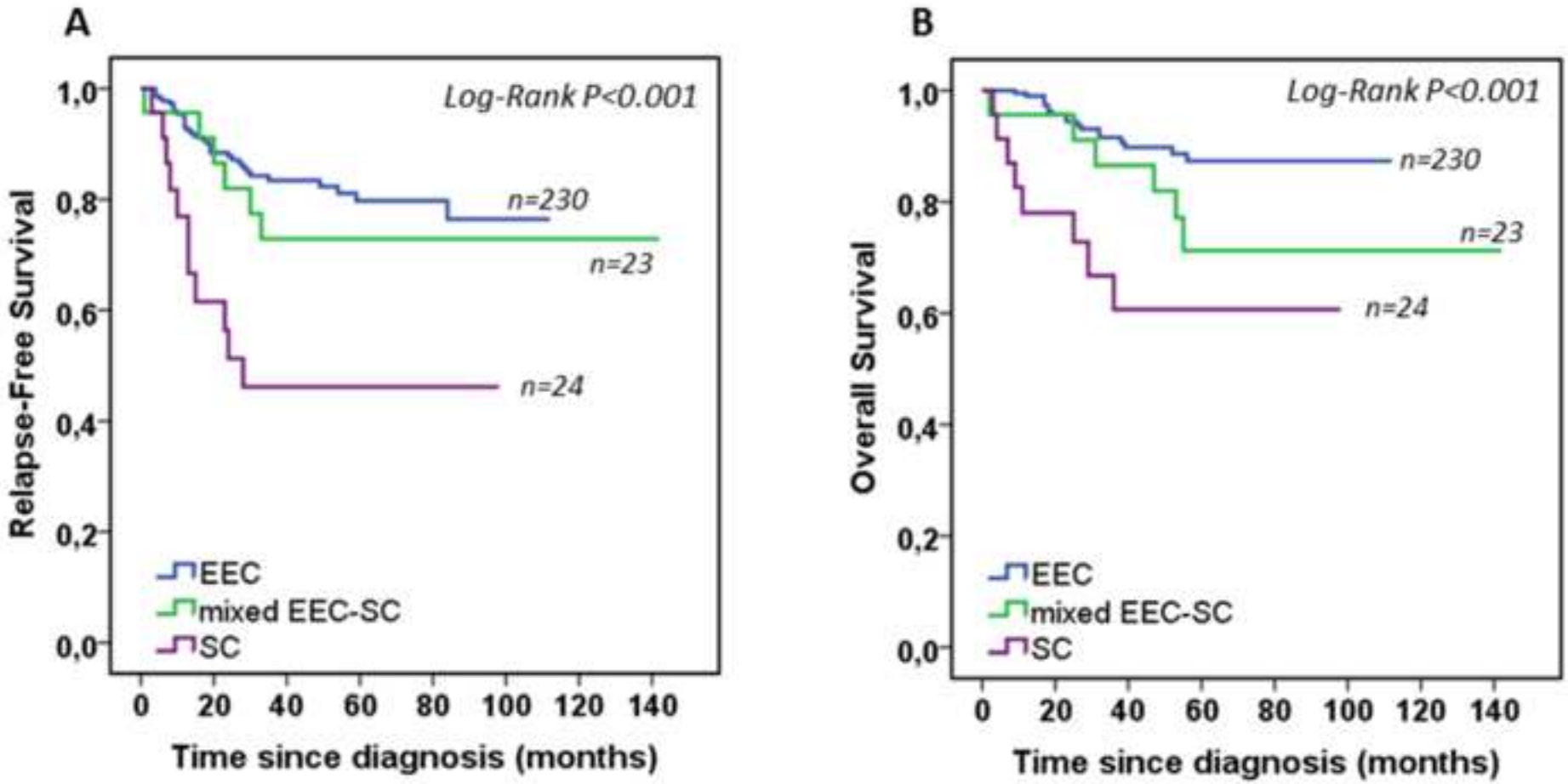


Figure
Click here to download high resolution image

Figure 2



Table

Table 1. Demographic and clinicopathologic characteristics of patients with pure EEC, mixed EEC-SC and pure SC.

Total cases analysed	Pure EEC n=230	Mixed EEC-SC n=23	Pure SC n=24	P value X^2 test
Age				
<60	57 (24.8%)	4 (17.4%)	9 (37.5%)	0.285
≥60	173 (75.2%)	18 (78.3%)	15 (62.5%)	
missing data	0	1 (4.3%)	0	
FIGO 2009				
I-II	178 (77.4%)	16 (69.6%)	13 (54.2%)	0.083
III-IV	52 (22.6%)	6 (26.1%)	10 (41.7%)	
missing data	0	1 (4.3%)	1 (4.1%)	
Lymph node involvement				
negative nodes	114 (49.6%)	15 (65.2%)	13 (54.2%)	0.307
positive nodes	29 (12.6%)	7 (30.5%)	6 (25.0%)	
missing data	87 (37.8%)	1 (4.3%)	5 (20.8%)	
Relapse				
no	191 (83.0%)	18 (78.3%)	12 (50.0%)	0.001
yes	34 (14.8%)	5 (21.7%)	11 (45.8%)	
missing data	5 (2.2%)	0	1 (4.2%)	
Follow up				
NED	194 (84.3%)	15 (65.2%)	11 (45.8%)	<0.001
AWED	7 (3.0%)	0	3 (12.5%)	
DID	8 (3.5%)	3 (13.1%)	0	
DOD	18 (7.8%)	5(21.7%)	8 (33.3%)	
missing data	3 (1.4%)	0	2 (8.3%)	

Clinical data are stratified for pure EEC, mixed EEC-SC and pure SC. Percentages are given as column %. P values of a Pearson's Chi Square test are given. Abbreviations: EEC, endometrioid endometrial carcinoma; mixed EEC-SC: mixed endometrioid and serous carcinoma, SC: Serous carcinoma; NED; no evidence of disease; AWED, alive with evidence of disease; DID, dead of intercurrent disease; DOD, dead of disease. Clinical variables were missing for some parameters and are indicated in the table.

Table 2: Cox Regression model proportional hazards (adjusted and unadjusted)

Relapse-Free survival	**Unadjusted hazard ratio**	**95% CI**	**P value**	**Adjusted hazard ratio**	**95% CI**	**P value**
Model 1						
Pure EEC	1			1		
Mixed EEC-SC	1.392	0.583-3.324	0.456	1.208	0.471-3.098	0.695
Pure SC	3.896	1.973-7.694	<0.001	3.444	1.721-6.894	<0.001
Model 2						
Pure EEC	0.257	0.130-0.507	<0.001	0.290	0.145-0.581	<0.001
Mixed EEC-SC	0.357	0.132-0.969	0.043	0.351	0.120-1.022	0.055
Pure SC	1			1		
Overall survival	**Unadjusted hazard ratio**	**95% CI**	**P value**	**Adjusted hazard ratio**	**95% CI**	**P value**
Model 1						
Pure EEC	1			1		
Mixed EEC-SC	2.358	0.932-5.968	0.070	1.970	0.726-5.345	0.183
Pure SC	4.831	2.099-11.116	<0.001	3.462	1.474-8.131	0.004
Model 2						
Pure EEC	0.207	0.090-0.476	<0.001	0.289	0.123-0.678	0.004
Mixed EEC-SC	0.488	0.169-1.415	0.187	0.569	0.182-1.777	0.332
Pure SC	1			1		

Cox regression analysis, whether or not adjusted for age and FIGO stage, was used to evaluate differences in RFS and OS in the three patient groups. In model 1, pure EEC was used as a reference, in model 2, pure SC was used as reference. Abbreviations: EEC: endometrioid endometrial cancer; SC: serous endometrial cancer.

Table 3. Number of pure EEC, mixed EEC-SC and pure SC carrying at least 1 mutation in the indicated genes.

	Pure EEC n=230	**mixed EEC-SC n=23**	**Pure SC n=24**	**P value X^2**
KRAS	39 (17.0%)	6 (26.0%)	3 (12.5%)	0.439
PIK3CA	31 (13.5%)	6 (26.0%)	4(16.7%)	0.258
TP53	4 (1.7%)	5 (22%)	4 (16.7%)	<0.001
PTEN	45 (19.6%)	3 (13.0%)	0	0.047

KRAS, *PIK3CA*, *PTEN*, and *TP53*, mutation frequencies respectively in pure EEC, mixed EEC-SC, and pure SC. Abbreviations: EEC, endometrioid endometrial carcinoma; Mixed EEC-SC: mixed endometrioid serous carcinoma; SC: Serous carcinoma. P values of a Pearson's chi square test are given.

Table 4. Hotspot mutation frequencies in separate components of mixed EEC-SC and pure EEC and pure SC.

	pure EEC	**EEC component in mixed EEC-SC**	**SC component in mixed EEC-SC**	**pure SC**
	n=230	**n=23**	**n=23**	**n=24**
KRAS	39 (17.0%)	5 (21.7%)	4 (17.4%)	3 (12.5%)
PIK3CA	31 (13.5%)	6 (26.0%)	6 (26.0%)	4 (16.7%)
TP53	4 (1.7%)	3 (13.0%)	4 (17.4%)	4 (16.7%)
PTEN	45 (19.6%)	3 (13.0%)	2 (8.7%)	0

KRAS, *PIK3CA*, *PTEN*, and *TP53*, mutation frequencies respectively in pure EEC, mixed EEC-SC, and pure SC. Abbreviations: EEC, endometrioid endometrial carcinoma; Mixed EEC-SC: mixed endometrioid serous carcinoma; SC: Serous carcinoma.

Supplementary Material

Click here to download Supplementary Material: Suplementary Tables.doc